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THE DIRECT DEVELOPMENT IN *RUNCINA SETOENSIS* BABA (OPISTHOBRANCHIA-CEPHALASPIDEA)[1]

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With Plates XXII–XXIV and 1 Text-figure

Runcina setoensis was established by BABA in 1954. The aim of this paper is to describe the breeding biology of the species in question, with the hope of giving some additional information concerning the direct development in the genus *Runcina* (cf. VAYSSIÈRE, 1900).

Nearly every autumn during the past several years there has been an enormous increase of this species on the coasts around the Seto Marine Biological Laboratory, Seto, Japan. As noted previously (BABA, 1954, p. 374), the animal prefers to live in the shallow tide-pools of high water regions (Pl. XXII, fig. 1), and it crawls about slowly on the bare bottom or on the bed of the young *Enteromorpha* weeds (Pl. XXII, fig. 2). In some cases the specimen is hidden underneath the pebbles. There are both small and larger individuals, the length varying from 3 mm to about 7 mm. No observations have yet been made on the feeding and spawning habits of this species in its native habitat.

Our thanks are due to Dr. Huzio UTINOMI of the Marine Biological Laboratory, who has given us facilities for studying the animal and for publishing the present paper.

Mrs. Ruth L. KOBS, M. A., a Fulbright instructor of English in our university, has kindly revised the English manuscript.

Spawning habits: *R. setoensis* may be kept alive in small glass vessels full of natural water. At first the newly collected animal from the sea does not lay eggs, and shows indication of trying to leave the water. It is after 6 to 7 days that the act of spawning takes place within the vessels. The following are the spawning data obtained by us in the laboratory.

The active season of spawning is from September until October, but it was not easy to breed this animal in captivity. The normal egg-masses contained from 50 to

1) Contributions from the Seto Marine Biological Laboratory, No. 327.

2) Grant in Aid for Developmental Scientific Research of the Ministry of Education.

Egg-masses No.	Collecting date	Spawning date	Egg number	Spawning temp.
I	IX-23-'57	IX-29	100	18°C
II	〃	〃	66	〃
III	〃	〃	25	〃
IV	X-20-'57	X-26	17	15°
V	〃	X-27	70	18°
VI	〃	X-28	21	〃
VII	〃	X-28	49	〃
VIII	〃	X-30	10	20°
IX	〃	X-31	18	／
X	I-6-'58	I-12	14	17-19°
XI	X-14-'58	X-19	2	／
XII	〃	X-22	12	21°
XIII	〃	X-26	65	19°
XIV	〃	X-30	2	／
XV	XI-24-'58	XI-28	2	15°
XVI	〃	XII- 5	15	〃

100 eggs, all deposited irregularly within a viscid mucous matter (Pl. XXII, figs. 3-4; Pl. XXIII, fig. 1). The approximate size of the egg-masses is from 3 mm² to 4 mm². In the glass vessels these egg-masses are found clinging to the inner wall of the vessels, just below the surface of the water. The spawning usually takes place early in the morning or late in the evening, the whole process requiring a comparatively long time.

Developmental changes: The egg-cells of *R. setoensis* thus laid pass into the adult form while they are within the egg-capsules (direct development). The developmental rate appears to be greatly affected by the temperature of the environment. The following is a rough table of developmental changes in the egg-masses, Nos. XII and XIII, during the months of October and November, at free room temperature.

2-cells to 8-cells ..... 1 day
8-cells to young morula..... 2-3 days
Primordial larval body ..... 4-5 〃
The earliest veligers ..... 6 〃
Rotation of veligers..... 7 〃
Mouth-slit opening in veligers.....14-15 〃
Shell and statocysts formation.....16-17 〃
Full-grown veligers .....18-19 〃
Metamorphosis .....20-26 〃
Intra-capsular adult form .....27 〃
Hatching .....38-42 〃

Egg-cells: In *R. setoensis* there appears a double membrane to each of the egg-capsules, and the outer layer is provided with fine striae on its surface. The diameter of the egg-capsules measures about 350μ; the intra-capsular space is roomy. The eggs are large, spherical, about 240-260μ across, and densely packed with deep orange-yellow yolk granules (Pl. XXII, fig. 5; Pl. XXIII, fig. 2). The maturation division occurs shortly after the eggs are laid.

Early cleavages: Quite normally, the early cleavages of the egg-cells continue, and the 8-cell stage is sometimes reached within the first day of development. The first cleavage is, as a rule, equal (Pl. XXII, fig. 6; Pl. XXIII, fig. 3), but sometimes it is unequal. At the 8-cell stage, each of the embryos consists of four micromeres which are translucent and colourless, and four macromeres which are opaque and deep orange-yellow (Pl. XXII, fig. 8; Pl. XXIII, fig. 4).

On the second or third day the micromeres proliferate to form a cap above the macromeres. The embryos at this stage are called the young morula (Pl. XXII, fig. 9; Pl. XXIII, fig. 5).

From this time on it has been difficult to follow accurately the further cleavages because of the richness of the yolk in the developing embryos. No formation of blastula and gastrula could be found by us. There is not a trochophore stage.

The embryos on the fourth and fifth days are tentatively named here as the primordial larval body (Pl. XXII, fig. 10; Pl. XXIII, fig. 6). On the outside they gradually become covered with fine cilia.

The earliest veligers: On the sixth day each of the primordial larval bodies shows the primitive features of a veliger (Pl. XXIII, fig. 7). The veliger's body in *R. setoensis* consists of: (1) a large spherical visceral sac, (2) a small protruding foot with a circular disc on the under side, and (3) a pair of velar ridges. The outer surface of the visceral sac is finely ciliated. Internally the visceral sac is packed with a yolky mass. The foot is colourless, having no cilia. The velar ridges with especially strong cilia are here referred to the velar lobes in the typical molluscan veligers.

Rotation of veligers: On the seventh day the veligers commence a rotatory movement (Pl. XXII, fig. 11), and this appears to be promoted by the strong cilia borne upon the velar ridges.

During the further growth of the veligers, from about the eighth to the eleventh day, the yolky mass of the visceral sac shows signs of being divided in two (Pl. XXII, fig. 12; Pl. XXIII, figs. 8 a-8 b). The whole integument of the body becomes clearly differentiated. It is at first colourless, but later it gets faintly yellow. On the foot-margin there appear a series of large refracting cells (mucous cells ?) among the epithelium, which soon becomes clothed with fine cilia.

Mouth-slit opening in veligers: On about the twelfth or the thirteenth day, the

rudiment of a mouth-slit first appears in the veligers as a collection of yellowish cells shining through between the two velar ridges (Pl. XXII, fig. 13; Pl. XXIII, fig. 9). On the following days, from the fourteenth to the fifteenth, the actual mouth-slit opens as a marked vertical slit (Pl. XXII, fig. 14; Pl. XXIII, figs. 10 a–10 b).

Shell and statocysts formation: As the veligers continue development, minute spots of chocolate-brown come over the visceral sac. An internal shell is gradually formed just above the posterior bifurcation of the liver. It is small, opaque, and white. Paired statocysts also arise at about the boundary between the visceral sac and the foot. This stage of development is seen on about the sixteenth or seventeenth day (Pl. XXIV, fig. 1).

Full-grown veligers: Apparently the veligers are fully grown on about the eighteenth or nineteenth day, and they largely occupy the inner space of each of the egg-capsules (Pl. XXII, fig. 15; Pl. XXIV, figs. 2 a-2 b). The rotation of the veligers still goes on. The main features observed in them at this stage may be summarized as follows: (1) A pair of marked velar ridges with strong cilia; (2) a large mouth-slit between the two velar ridges; (3) the paired statocysts; (4) an internal shell; and (5) an expanded pedal disc. The general integument, including the pedal disc, is ashy yellow, and shows an increasing number of chocolate-brown spots on the visceral sac. Externally, the whole integument is finely ciliated. There are two, well-defined liver-lobes; these are symmetrical, of nearly equal size, yolky, and deep orange-yellow in colour.

Metamorphosis: After fully grown, the veligers tend to be still and inactive in rotation. It is during the days from about the twentieth to the twenty-sixth that the transformation takes place in certain organs of these embryos (Pl. XXII, fig. 16; Pl. XXIV, figs. 3 a–3 b). The most notable is the degeneration and final absence of the velar ridges; the next is the decreasing in size of the mouth-slit. Against the reducing tendency of these structures, there will arise an indication of the anus just below the shell. The pedal disc widens still more. A thicker distribution of the chocolate-brown spots is observed on the visceral sac; these spots may also be seen on the foot-margin. At about the close of the metamorphosis there appears black pigment on the visceral sac (Pl. XXIV, fig. 4), and this gradually covers the ashy yellow ground-colour of the mantle. The veligers which have undergone metamorphosis now assume the general appearance of an adult.

Intra-capsular adult form: The metamorphosed embryos remain within each of the egg-capsules still longer, and very slowly they keep on rotating (Pl. XXIV, figs. 5 a–5 b). On about the twenty-seventh day the embryos appear to be more or less elongated: there are no velar ridges; the black pigment prevails on the mantle-surface; clusters of black spots mark the head-region on each side; the mouth-slit is decreased; the shell denotes the posterior end of the mantle; the anus clearly opens

below the shell. The chocolate-brown spots are thick on the visceral sac, but less so on the pedal disc. The pedal disc widens noticeably, with the entire margin curving upward. The black pigmentation appears on the foot-margin and around the anus. The paired liver-lobes decrease in size.

The succeeding development is only gradual. During this period the somewhat enlarged and lengthened embryos appear to roll up in the limited space of the egg-capsules (Pl. XXII, fig. 17; Pl. XXIV, figs. 6 a–6 b). The embryos are now prepared to hatch.

Hatching: From spawning to hatching, the whole development of the embryos takes from about 38 to 42 days. At the time of hatching the egg-capsules are swollen

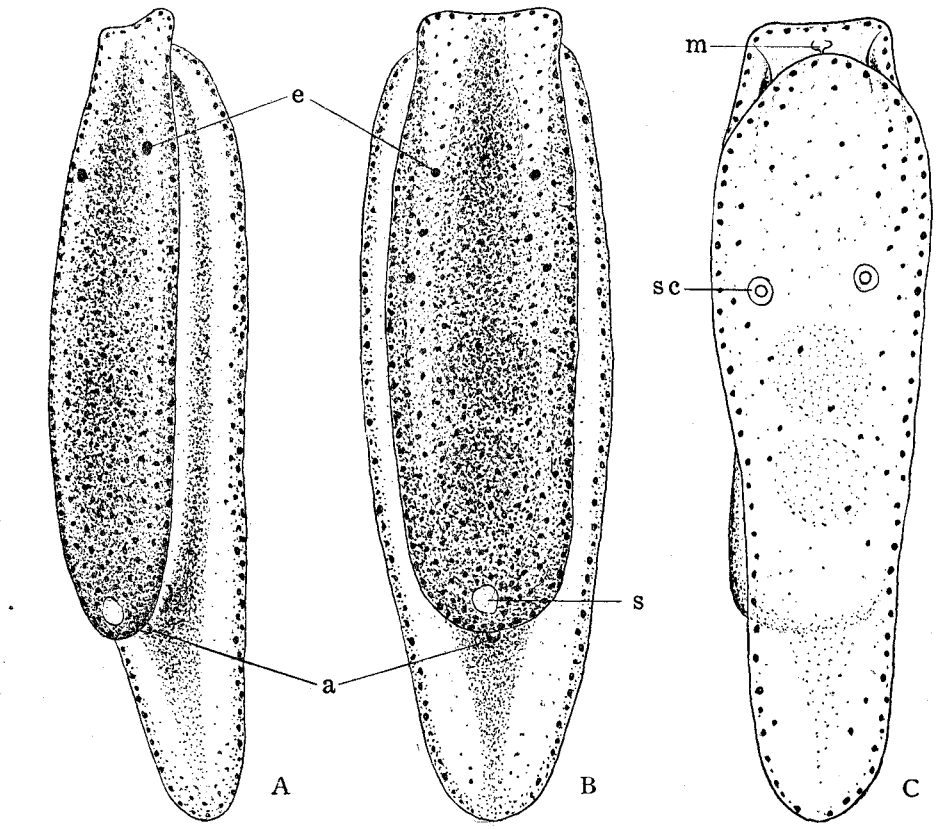

Fig. 1. The young individuals of *Runcina setoensis*, immediately after hatching. L. 600μ.
A. lateral, B. dorsal, C. ventral. a. anus, e. eye, m. mouth, s. shell, sc. statocyst.

for easy bursting of the wall; the embryos are thus allowed to pass out in full extension (Pl. XXII, fig. 18; Pl. XXIV, figs. 7 a–7 b).

As previously stated, these embryos are much like the adult in general form and

in colouring (Pl. XXII, fig. 19; Text-fig. 1, A–C). The total length of them measures about 600μ. The whole embryo consists of the visceral sac or the mantle, the sides, and the foot. It is finely ciliated everywhere.

The ground-colour of the integument is ashy yellow. On the mantle the richly distributed chocolate-brown spots become almost hidden by the prevailing black pigment, which is thickest in the median part and around the margin. A pair of black-pigmented eyes is in the head-region. A few additional black spots may occur near each of the eyes. Deep orange-yellow liver-lobes, greatly reduced in size, lie within the posterior half of the visceral sac. At the posterior end of the mantle is a small, opaque, white, internal shell. Just below the shell opens the anus, which is marked by a black border. A deep black band is formed on each side of the body, on foot-margins, and on the tail in the middle line. A small, not black-pigmented mouth-slit appears on the under side of the head. The pedal disc is ashy yellow, with chocolate-brown spots that tend to be arranged in a series near the margins. A pair of statocysts is visible through the transparent sole.

The young individuals were kept alive for two weeks more, and then killed. They attained the length of about 700μ. The gills, the genital orifices, and the genital groove were not well developed at that time.

Summary, with short discussions

1. The first record of the direct development in the genus *Runcina* is that of *R. coronata* (QUATREFAGES) by VAYSSIÈRE, 1900, but the accounts given by him are inadequate. The second recording is here made by us for *R. setoensis* BABA. As to the species of the direct development in the order Opisthobranchia, see BABA, 1937.

2. At Seto, Japan, the species *setoensis* appears on shores abundantly from September to November, and the most active season for spawning is during September and October. The individuals are 3–7 mm in length.

3. The spawning has been observed in the laboratory glass vessels only. The egg-masses have no definite shape as in *R. coronata*, and normally contain 50–100 eggs.

4. The egg-cells are very large compared to the size of the parent, being 240–260μ across, thickly yolk-laden, and deep orange-yellow in colour. The eggs in many opisthobranchs are more or less than 100μ. In *R. setoensis* each of the egg-capsules has a double membrane.

5. During October and November at free room temperature, it took from about 38 to 42 days for the whole development within the egg-capsules. At the close of this period the embryos, nearly in an adult form, became free.

6. The earliest cleavages are approximately as usual. It was hardly possible to determine the formation of the blastula and the gastrula accurately. There was no trochophore stage. The primordial larval bodies are covered with fine cilia, and these pass into the veliger stage.

7. The veligers of *R. setoensis* are very much modified, but they may be called as such if we consider the strongly ciliated velar ridges of *setoensis* to be similar in function to the velar lobes in many molluscan veligers.

8. The veliger's body consists of a large spherical visceral sac and a circular pedal disc. During the formation of the mouth-slit between the two velar ridges, there arise a pair of statocysts and an internal shell. No external nautiloid shell or operculum was seen during the course of the development. The veligers are in active rotation.

9. The metamorphosis which occurs at about the middle of the whole development is shown by the degeneration of the velar ridges. The mouth-slit, which lies in front, decreases in size. The anus, found on the posterior side of the embryo, begins to open just below the shell.

10. Next comes the intra-capsular adult form. The body elongates, the black pigment develops on the visceral sac, and the embryos await hatching. The newly freed individuals are about 600μ long.

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Postscript

A brief observation on the direct development of *Runcina elioti* BABA was made at Seto by HAMATANI in January, 1958 (spawning on Jan. 9th).

EXPLANATION OF PLATES XXII–XXIV

PLATE XXII

(Figs. 5–17, ×100)

Fig. 1. Collecting of *Runcina setoensis* from the tide-pool zone near the Seto M. B. L. (IX–'57).

2. *R. setoensis* in the native habitat (X–'58). L. 7 mm.

3. The egg-mass No. II, deposited in the laboratory glass vessel (IX–'57). ×7.

4. Part of the egg-mass. ×25.

5. Newly laid egg-cell in maturation division. Same figure as Pl. XXIII, fig. 2.

6. 2-cells. Same figure as Pl. XXIII, fig. 3.

7. 4-cells.

8. 8-cells on the second day. Roughly the same figure as Pl. XXIII, fig. 4. Four micromeres at the animal and four macromeres at the vegetal pole.

9. Young morula on the third day. Roughly the same figure as Pl. XXIII, fig. 5. Cap of micromeres at the animal pole.

10. Primordial larval body on the fifth day. Fine ciliation on the whole surface. A day later than Pl. XXIII, fig. 6.

11. Earliest veliger on the seventh day, from the posterior side. Commencement of rotation. A spherical visceral sac above, and a small protruding foot below. A day later than Pl. XXIII, fig. 7.

12. Veliger on the ninth day, from the anterior side. Roughly the same figure as Pl. XXIII, fig. 8 a.

13. Veliger on the twelfth day, from the posterior side. Formation of the rudiment of a mouth-slit. Same stage as Pl. XXIII, fig. 9.

14. Veliger on the fifteenth day, from the anterior side. Actual opening of the mouth-slit. Roughly the same figure as Pl. XXIII, fig. 10 a.

15. Full-grown veliger on the eighteenth day, from the postero-lateral side. Roughly the same figure as Pl. XXIV, fig. 2 b.

16. Veliger in metamorphosis on the twenty-second day. The visceral sac above, and the pedal disc below. Roughly the same stage as Pl. XXIV, fig. 3 a.

17. Intra-capsular adult form on the twenty-eighth day, from the left side. Roughly the same figure as Pl. XXIV, fig. 6 b.

18. Hatching of the young individuals (×35) on about the forty-second day. Roughly the same figures as Pl. XXIV, figs. 7 a–7 b.

19. A newly hatched individual from above. L. 600μ. Same figure as text-fig. 1, B.

PLATE XXIII

(Figs. 2–10 b, ×120)

Fig. 1. The egg-mass No. V, deposited in the laboratory glass-vessel (X-'57). ×17.

2. Newly laid egg-cell in maturation division. Egg diameter 240–260μ.

3. 2 cells.

4. 8 cells. Four micromeres at the animal and four macromeres at the vegetal pole.

5. Young morula on the third day. Cap of micromeres at the animal pole. With 3 polar bodies above the cap.

6. Primordial larval body on the fourth day. Fine ciliation begins to develop on the surface.

7. Earliest veliger on the sixth day, from the posterior side. Showing the visceral sac above, and the small protruding foot below.

8 a. Veliger in rotation on the ninth day, from the anterior side. Showing a pair of strongly ciliated velar ridges.

8 b. The same, from the posterior side.

9. Veliger on the twelveth day, from the anterior side. Formation of the rudiment of a mouth-slit.

10 a. Veliger on the fifteenth day, from the anterior side. Actual opening of the mouth-slit between the two velar ridges. Liver clearly divided in two. Chocolate-brown spots appearing on the visceral sac.

10 b. The same, from the posterior side.

PLATE XXIV

(Figs. 1–7 b, ×120)

Fig. 1. Veliger on the sixteenth day, from the posterior side. Showing the formation of a shell and paired statocysts.

2 a. Full-grown veliger on the eighteenth day, from the anterior side.

2 b. The same, from the posterior side.

3 a. Veliger in metamorphosis on the twenty-second day, from the right side.

3 b. The same, from the anterior side.

4. Veliger on the twenty-fifth day, from the anterior side. At about the close of the metamorphosis. The velar ridges are about to disappear entirely; the highly decreased mouth-slit is almost invisible between the visceral sac and the foot.

5 a. Intra-capsular adult form on the twenty-seventh day, from the anterior side. Black pigmentation prevails over the visceral sac.

5 b. The same, from the posterior side. The anal opening clearly visible just below the shell.

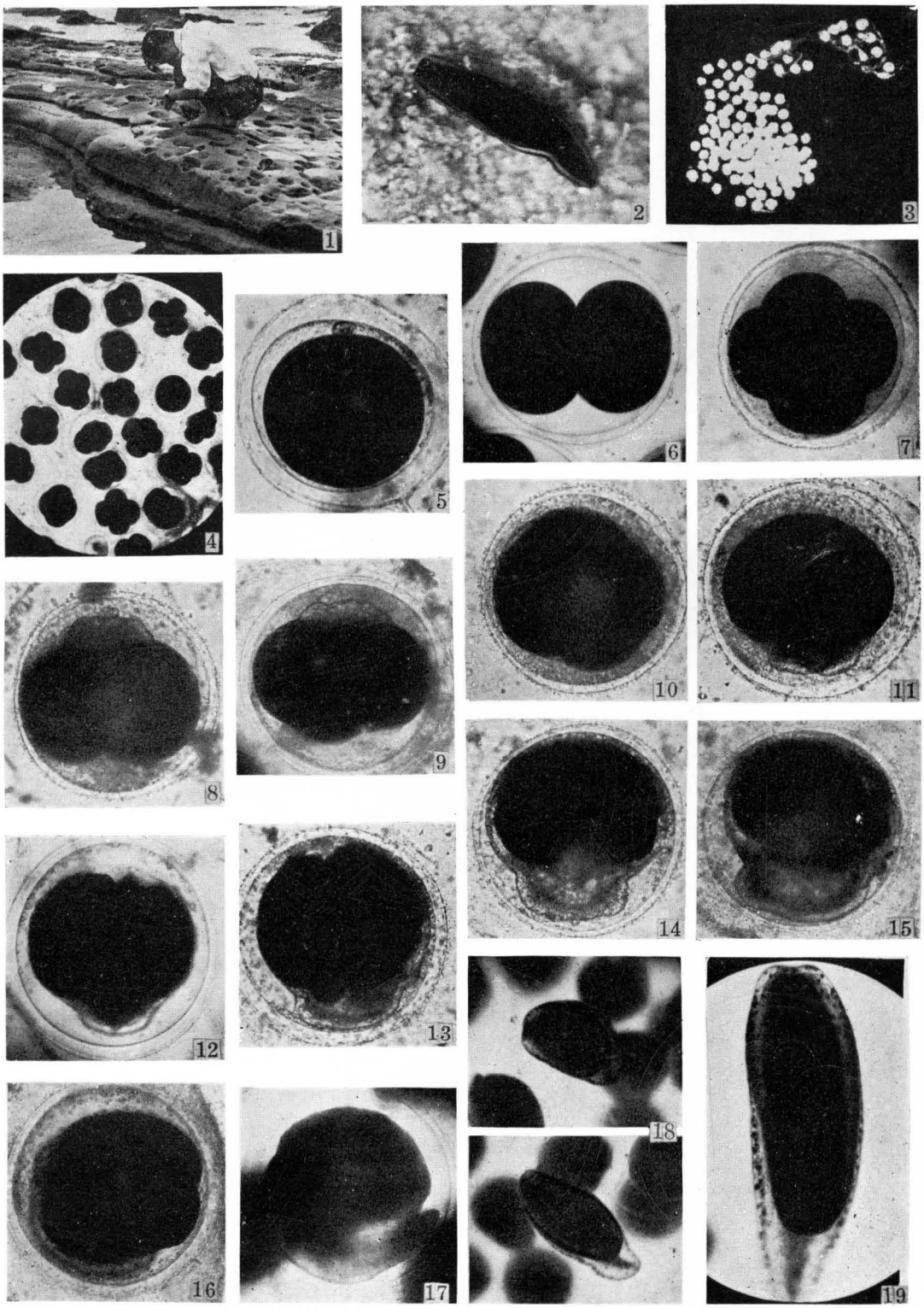
6 a. The intra-capsular adult form on the thirty-second day, slightly from the antero-lateral side. The enlarged and lengthened embryo appearing to roll up within the egg-capsule. With an exceedingly expanded pedal disc.

6 b. The same, slightly from the postero-lateral side.

7 a. Hatching of the young individual on about the forty-second day, from the right side.

7 b. The same, from the posterior side.

Abbreviations: A. anus; m. mouth-slit; p. foot or pedal disc; s. shell; sc. statocyst; vr. velar ridge; vs. visceral sac. Anterior side=oral side. Posterior side=anal side.



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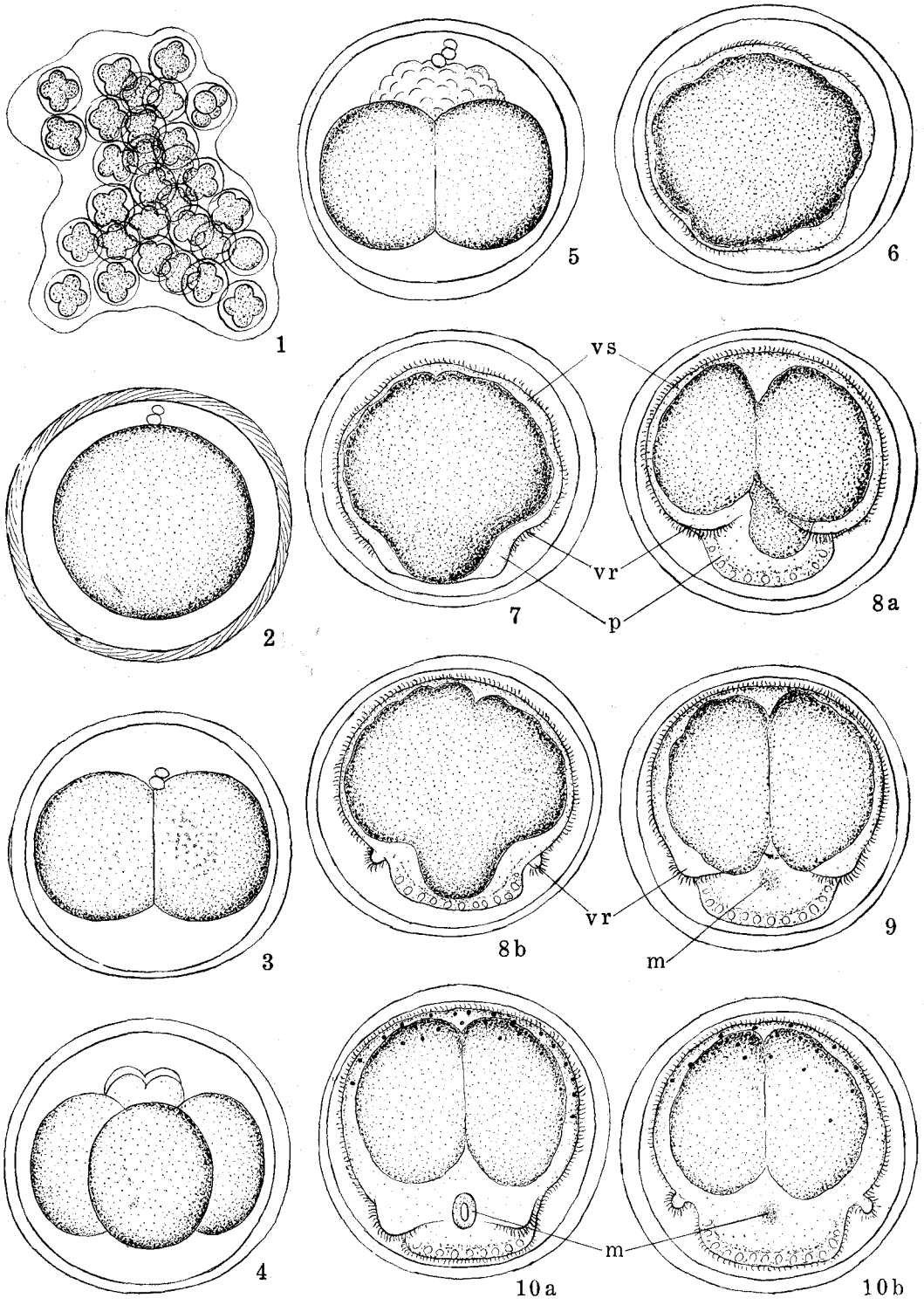

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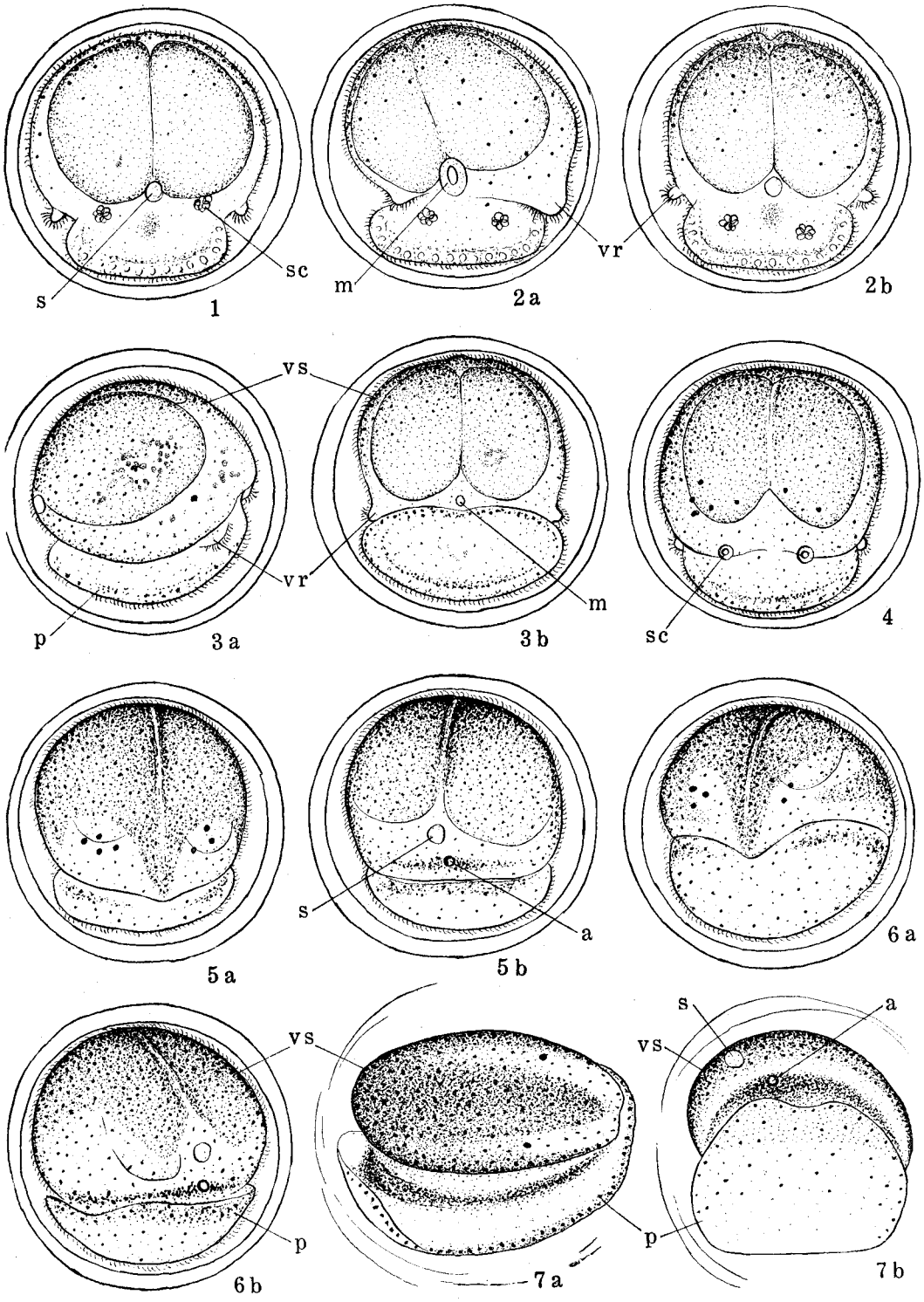

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